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Human Metapneumovirus Infection in Chronic Obstructive Pulmonary Disease: Impact of Glucocorticosteroids and Interferon

Keiko Kan-o,[1,2,3,7] Ruben Ramirez,[4] Martin I. MacDonald,[1] Michael Rolph,[5] Penny A. Rudd,[5] Kirsten M. Spann,[6] Suresh Mahalingam,[5] Philip G. Bardin,[1,2,3,a] and Belinda J. Thomas[1,2,3,a]

[1]Monash Lung & Sleep, Monash Medical Centre, [2]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, and [3]Department of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Melbourne, [4]Faculty of Applied Science, University of Canberra, [5]Institute for Glycomics, Griffith University, Southport, and [6]School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia; [7]Research Institute for Disease of the Chest, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Background. Human metapneumovirus (hMPV) infection is implicated in exacerbations of asthma and chronic obstructive pulmonary disease (COPD). Research into the pathogenesis of infection is restricted to animal models, and information about hMPV replication and inflammatory and immune responses in human disease is limited.

Methods. Human primary bronchial epithelial cells (PBECs) from healthy and asthmatic subjects and those with COPD were infected with hMPV, with or without glucocorticosteroid (GCS) exposure. Viral replication, inflammatory and immune responses, and apoptosis were analyzed. We also determined whether adjuvant interferon (IFN) can blunt hMPV infection in vitro and in a murine model.

Results. hMPV infected human PBECs and viral replication was enhanced in cells from patients with COPD. The virus induced gene expression of IFN-stimulated gene 56 (ISG56) and IFN-β, as well as IFN-γ-inducible protein 10 (IP-10) and regulated on activation, normal T cell expressed and secreted (RANTES), and more so in cells from patients with COPD. GCS exposure enhanced hMPV replication despite increased IFN expression. Augmented virus replication associated with GCS was mediated by reduced apoptosis via induction of antiapoptotic genes. Adjuvant IFN treatment suppressed hMPV replication in PBECs and reduced hMPV viral titers and inflammation in vivo.

Conclusions. hMPV infects human PBECs, eliciting innate and inflammatory responses. Replication is enhanced by GCS and adjuvant IFN is an effective treatment, restricting virus replication and proinflammatory consequences of hMPV infections.

Keywords. human metapneumovirus; human primary bronchial epithelial cells; glucocorticosteroids; interferon; apoptosis; innate immune response.

Human metapneumovirus (hMPV) was discovered only in 2001, as a member of the Paramyxoviridae family, and it is closely related to respiratory syncytial virus (RSV) [1, 2]. hMPV causes acute lower respiratory infection in children, the elderly, and immunocompromised patients, but it has also been implicated in exacerbations of asthma and chronic obstructive pulmonary disease (COPD), although studies have been limited [3–6]. There is currently no vaccine or therapy available for hMPV.

Clinical studies have demonstrated that hMPV is a viral pathogen associated with respiratory infections that in patients with COPD lead to exacerbations, hospitalizations, and use of oral corticosteroids [3, 4]. Both animal and human in vivo studies have confirmed that airway epithelium can sustain hMPV infection and replication [7, 8]. After infection, viral RNA is recognized by various pattern recognition receptors, leading to the secretion of type I interferons (IFNs) and proinflammatory cytokines and chemokines. Type I IFNs stimulate neighboring cells to express IFN-regulated genes (IRGs), inducing an antiviral state. Although it has been shown by other investigators that hMPV can replicate and induce the production of inflammatory cytokines, chemokines, and type I IFNs in human epithelial cell lines [9], there have been no reports describing hMPV infection of primary culture bronchial epithelial cells obtained from patients with diseases such as asthma or COPD. Importantly, it is unknown whether hMPV infection in asthma and COPD is associated with impaired host innate immune responses to hMPV as demonstrated for rhinovirus (RV) and influenza virus infection [10, 11].

The benefits of inhaled glucocorticosteroids (GCSs) are widely recognized. However, a detrimental effect of inhaled

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[a]P. G. B. and B. J. T. contributed equally to this work.

Correspondence: B. J. Thomas, PhD, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, 27–31 Wright St, Clayton, Victoria 3168, Australia (Belinda.thomas@monash.edu).

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GCSs in clinical medicine has been reported, its use being associated with an increased incidence of pneumonia in COPD and possibly also in asthma [12, 13]. Moreover, GCSs may offer little protection against virus-induced exacerbations, because anti-inflammatory benefits may be offset by detrimental effects on immune responses [14, 15]. We have shown in an animal model that although GCS treatment blunted inflammation, it also significantly reduced the expression of innate antiviral genes, with increased replication of RV and influenza A and more severe overall lung disease [16]. These adverse consequences of GCS could be reversed by administration of recombinant IFN [16]. It is unknown whether similar responses pertain to hMPV infections.

In the current study, we have evaluated the infection profile and immune responses to hMPV infection in primary bronchial epithelial cells (PBECs) obtained from adult volunteers who were either healthy or had asthma or COPD. We also assessed the effect of GCS on hMPV replication and immune responses and examined whether recombinant IFN attenuates hMPV infection both in vitro and in vivo.

METHODS

Virus Detection in Exacerbations of COPD

To examine the association of hMPV infections with COPD exacerbations, consecutive patients experiencing an exacerbation of COPD and admitted to hospital were recruited to a prospective observational study approved by Monash Medical Centre Human Research Ethics Committee (HREC 13134A). All patients had a nasopharyngeal swab taken, and viruses were identified by means of respiratory multiplex polymerase chain reaction (AusDiagnostics Highplex).

Culture of PBECs

PBECs were obtained during routine fibreoptic bronchoscopy from normal volunteers, asthmatics, and patients with COPD. Definitions of healthy volunteers and patients are described in detail in the Supplementary Methods. The baseline characteristics of patients from whom epithelial brushings and cells for in vitro studies were obtained are shown in Supplementary Table 1. Studies were approved by Monash Health Human Research Ethics Committee (HREC 07025A), and written informed consent was obtained. PBECs were obtained from bronchial brushings of the left or right upper lobe (away from any lung abnormalities if present) and cultured under submerged conditions, as described elsewhere [16].

Virus Infection of Primary Culture Cells

The CAN97-83 strain of hMPV was used in all experiments, propagated in LLC-MK2 cells, and viral titer was determined by means of median tissue culture infective dose assay, as described elsewhere [17]. This strain of hMPV has been used in other studies [18], and we did not attempt further purification because this may change key properties of the virus [19]. Control experiments using UV-inactivated hMPV were included to confirm that responses were a result of hMPV infection and not from factors remaining in the culture media after hMPV propogation. Infection protocols and GCS and IFN treatments are described in detail in the Supplementary Methods.

Gene Expression in Human Airway Cells

Total RNA was isolated from PBEC lysates and used for real-time polymerase chain reaction, as described in the Supplementary Methods. Primer sequences were as described elsewhere [16, 20] or are provided in Supplementary Table 2.

Detection of Human Cytokines and Chemokines

Cytokine levels in cell supernatants were determined using a Cytometric Bead Array Human Chemokine Kit (BD Biosciences; detection limit, 0.2–2.8 pg/mL). Interleukin 6 (IL-6) was measured by means of enzyme-linked immunosorbent assay (R&D Systems; detection limit, 9–38 pg/mL).

Caspase Assay

Caspase 3/7 activities in PBECs were measured using a Caspase-Glo 3/7 assay (Promega), according to the manufacturer's instructions.

Cell Apoptosis Assay

Annexin V binding was performed on PBECs using a fluorescein isothiocyanate annexin V Apoptosis Detection Kit (Biolegend). Briefly, cells were rinsed with cell staining buffer, resuspended in annexin V binding buffer with fluorescein isothiocyanate –conjugated annexin V and propidium iodide (PI) for 15 minutes, and then analyzed with flow cytometry.

hMPV Infection in a Murine Model

BALB/c mice were purchased from the Animal Resource Centre and housed under pathogen-free conditions at the University of Canberra (Australia). All procedures were approved by the University of Canberra Animal Ethics Committee and conducted following the Animal Welfare Guidelines of the National Health and Medical Research Council of Australia.

Mice were infected intranasally with 10^6 plaque-forming units of hMPV [21]. At 1 and 3 days after infection, mice were anaesthetized and given intratracheal instillation of recombinant murine IFN-β (R&D Systems) or vehicle (0.1% bovine serum albumin/phosphate-buffered saline). Mice were killed 5 days after infection, and lung tissue was processed to calculate viral titers and quantify pulmonary inflammation. Methods to quantify changes on lung histology are described in the Supplementary Methods.

Statistical Analyses

Unless otherwise stated, values are expressed as means with standard errors of the mean. Unpaired *t* tests were used to

compare of 2 sets of data. When comparing >2 sets of data, analyses were conducted using 1- or 2-way analysis of variance. Correlations were examined using Spearman correlation. All data analyses were conducted using GraphPad Prism 6 software (GraphPad Software). Results were considered statistically significant at $P \leq .05$.

RESULTS

hMPV Infection Associated With COPD Exacerbations

To evaluate detection of hMPV during COPD exacerbations, nasopharyngeal samples were obtained at admission in 167 patients (mean age, 71 years). Viruses were detected in 43 of 167 (26%), and, as expected, RV was the virus most frequently identified. Notably, hMPV was found in 8 of 167 patients (5% overall) and was the second most common virus species identified (8 of 44; 18%) (see Supplementary Figure 1).

hMPV Replicates in and Elicits Antiviral Responses From PBECs

hMPV infection has been described in human nasal and tracheal epithelial cells [18], but there are no reports of PBEC infection to our knowledge. Using PBECs isolated from normal volunteers, asthmatics, and patients with COPD, we evaluated infection profiles and immune responses to infection. hMPV replication was investigated after infection with hMPV at a multiplicity of infection (MOI) of 1. In PBECs from all groups, replication increased over a 72-hour time course, as evidenced by viral titration and an increase in hMPV nucleocapsid (hMPV N) gene expression. Viral titration of cell culture supernatants demonstrated robust hMPV replication, higher in cells from patients with COPD (Figure 1A). hMPV N gene expression was significantly higher in PBECs from patients with COPD (Figure 1B). The increase in hMPV N gene expression was absent if the virus was UV irradiated, thereby excluding non-hMPV induced responses (see Supplementary Figure 2*A*).

Increases in hMPV replication were accompanied by significant increases in ISG56 and IFN-β gene expression, which was again higher in PBECs from COPD (Figure 1C). The specificity of this response was verified, because UV-irradiated hMPV did not induce ISG56 gene expression (Supplementary Figure 2*B*).

Next, we examined hMPV-induced proinflammatory cytokine and chemokine responses. No evidence of IL-6 and interleukin 8 (IL-8) release above control levels was noted, but hMPV induced IFN-γ-inducible protein 10 (IP-10) in all 3 groups (Figure 1D and data not shown). Regulated on activation, normal T cell expressed and secreted (RANTES) production was significantly increased only in PBECs from patients with COPD (Figure 1D). Monocyte chemotactic protein 1 (MCP-1) and monokine induced by IFN-γ (MIG) production by hMPV infection was not observed (data not shown). UV-irradiated hMPV did not induce IP-10 and RANTES production (Figure 1D and Supplementary Figure 2*C*). hMPV N, ISG56, and IFN-β gene expression and cytokine and chemokine patterns of release at an MOI of 1 were similar if an MOI of 0.1 was used (see Supplementary Figure 3).

Effect of GCS on hMPV Replication and hMPV-Induced Antiviral Responses in PBECs

Because GCSs are the mainstay of current treatment for patients with asthma and in a significant number of those with COPD, we assessed the effects of GCS on hMPV replication and innate antiviral and inflammatory responses. PBECs from healthy subjects were treated with 10 nmol/L fluticasone propionate (FP) for 24 hours before infection with hMPV. This concentration of FP has been shown to be reflective of therapeutic levels achieved during inhalation in human lung [16, 22, 23].

GCS (FP) significantly increased viral shedding into cell supernatants at 72 hours after infection and hMPV N gene expression at 48 and 72 hours after infection (Figure 2A). To confirm that the increase in hMPV was due to GCS, mifepristone (RU486), a steroid receptor antagonist, was used as a control and showed that blocking the steroid receptor normalized hMPV replication (Figure 2A).

We examined myxovirus resistance protein 1 (MxA) and 2',5'-oligoadenylate synthetase (2'5'OAS) as representative IRGs responsive to infections. Unexpectedly, GCS also significantly up-regulated these hMPV-induced IRGs and IFN at 48 hours after infection (Figure 2B). A linear association was noted between hMPV N and ISG56 gene expression using data generated at 48 and 72 hours after infection in untreated cells. This link was not altered by GCS (Figure 2C), suggesting appropriate IFN responses over time to increasing virus replication. In line with our previous study, GCS treatment by itself had no effect on IRG and IFN gene expression (data not shown) [16]. GCS had similar effects on hMPV replication and antiviral responses when cells were infected at a lower MOI of 0.1 (see Supplementary Figure 4).

The effect of GCS on the expression of proinflammatory cytokines and chemokines after hMPV infection of PBECs was examined. GCS treatment before infection had no effect on IL-6 and IL-8 production, with levels similar to hMPV infection alone (data not shown). However, IP-10 gene expression was reduced at 48 hours, and protein levels were suppressed at 72 hours in response to GCS (Figure 2D). RANTES can be induced by IFN, and gene expression increased at 72 hours after infection, conceivably as a result of increases in IFN-β and ISG56 (Figure 2D and Figure 1C). GCS treatment alone had no effect on cytokine production (data not shown) [16].

Effect of GCS on Apoptosis in PBECs

Our studies using RV and IAV have found increased virus replication in response to GCS that was coupled to blunted IFN responses [16]. It was therefore surprising that IFN responses were robust in the context of enhanced hMPV

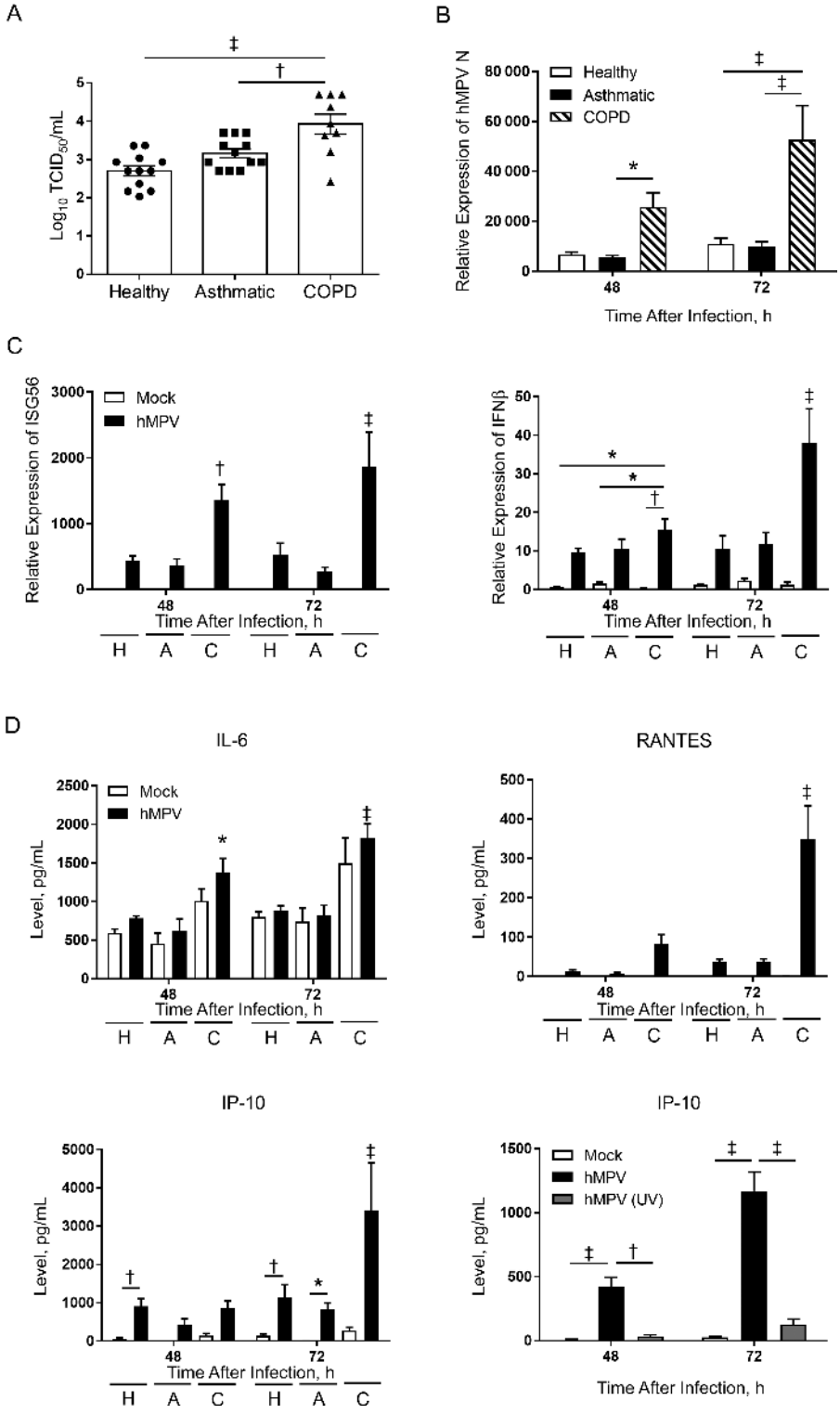

Figure 1. Human metapneumovirus (hMPV) replicates and induces antiviral and inflammatory responses in human primary bronchial epithelial cells (PBECs) from healthy individuals and patients with asthma or chronic obstructive pulmonary disease (COPD). PBECs were infected with hMPV (multiplicity of infection [MOI], 1). *A*, Viral titers of culture supernatants at 72 hours after infection were determined by means of plaque assay. $TCID_{50}$, median tissue culture infective dose. *B, C,* RNA was extracted from cell monolayers at 48 or 72 hours after infection, and reverse-transcription polymerase chain reaction was performed. Gene expression of hMPV nucleocapsid (hMPV N) (*B*), interferon (IFN)-stimulated gene 56 (ISG56), and IFN-β (*C*) was determined and normalized to 18S. *D*, Proinflammatory cytokine and chemokine levels in culture supernatants were determined using a Cytometric Bead Array Human Chemokine Kit (BD Biosciences) or enzyme-linked immunosorbent assay. H, healthy; A, asthmatic; C, COPD. Data represent means with standard errors of the mean for 3 replicates from ≥3 independent donors. PBECs from patients with COPD, when infected with hMPV, had significantly elevated interleukin 6 (IL-6) levels compared with all other hMPV-infected groups and significantly elevated regulated on activation, normal T cell expressed and secreted (RANTES) and IFN-γ-inducible protein 10 (IP-10) levels compared with all other groups. Comparisons were analyzed using 1- or 2-way analysis of variance, as appropriate. $^{*}P < .05$; $^{\dagger}P < .01$; $^{\ddagger}P < .001$.

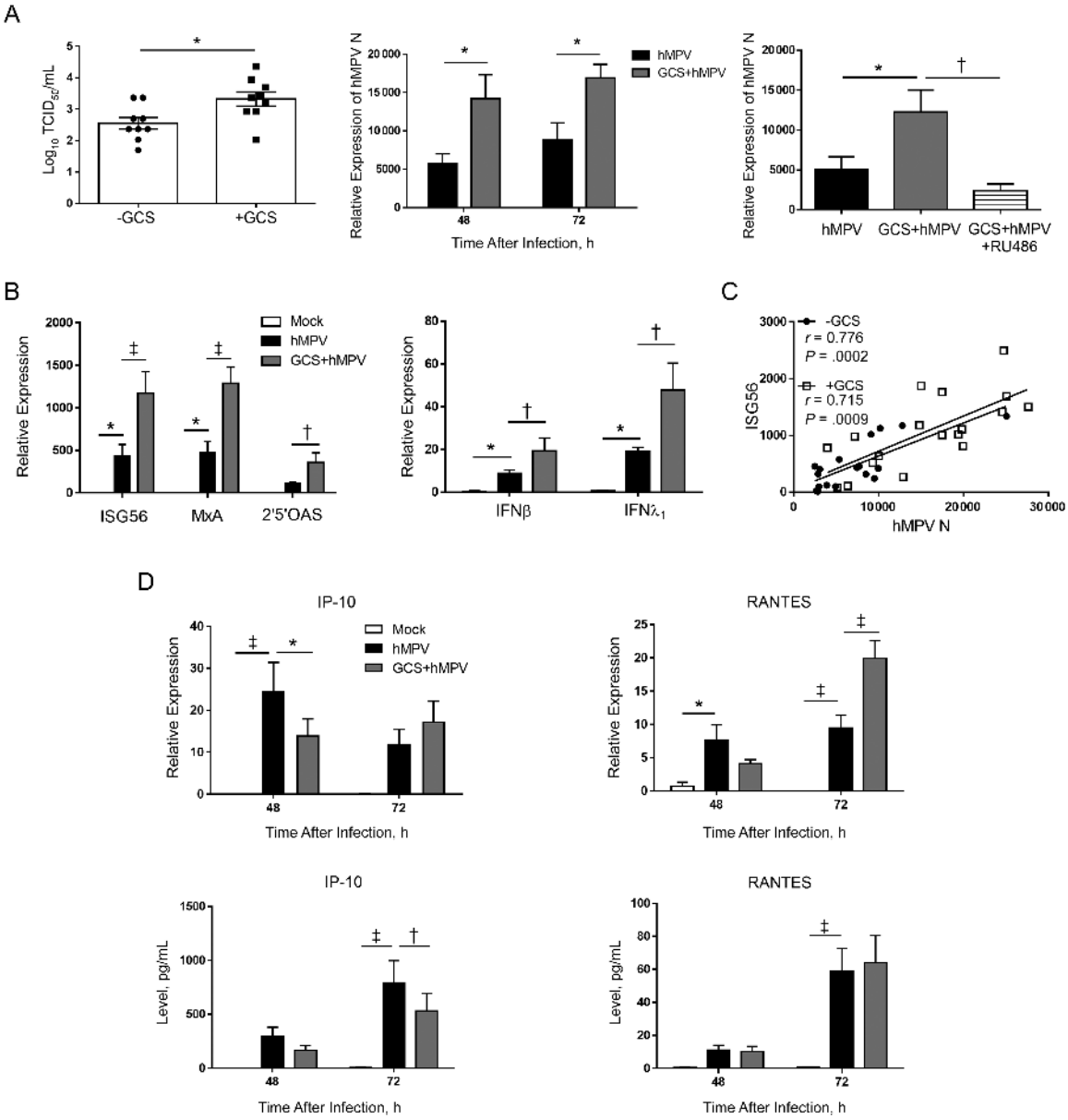

Figure 2. Glucocorticosteroid (GCS) treatment enhances human metapneumovirus (hMPV) replication, and hMPV-induced innate antiviral responses in human primary bronchial epithelial cells (PBECs) from healthy individuals. PBECs were pretreated with or without GCS and then infected with hMPV (multiplicity of infection, 1). RU486 was added before and after infection. RNA was extracted from cell monolayers at 48 or 72 hours after infection, and reverse-transcription polymerase chain reaction was performed. *A*, Viral titers of culture supernatants at 72 hours after infection were determined by means of plaque assay; there was no significant difference between hMPV and GCS+hMPV+RU486 samples (*right panel*). $TCID_{50}$, median tissue culture infective dose. Gene expression of hMPV nucleocapsid (hMPV N) (*A*), interferon (IFN)–regulated genes (IRGs) and IFNs (*B*), and IFN-γ-inducible protein 10 (IP-10) and regulated on activation, normal T cell expressed and secreted (RANTES) (*D*) were measured and normalized to 18S. MxA, myxovirus resistance protein 1; 2′5′OAS, 2′,5′-oligoadenylate synthetase. *C*, Correlation between gene expression of hMPV N and IFN-stimulated gene 56 (ISG56) at 48 and 72 hours after infection. *D*, IP-10 and RANTES protein levels in culture supernatants were determined using a Cytometric Bead Array Human Chemokine Kit (BD Biosciences). Data represent means with standard errors of the mean for 3 replicates from ≥3 independent donors. Comparisons were analyzed using *t* tests (viral titers), Spearman correlation, and 1- or 2-way analysis of variance as appropriate. **P* < .05; †*P* < .01; ‡*P* < .001.

replication, and we investigated inhibition of apoptosis as an alternative mechanism whereby GCS may exert detrimental effects. First, flow cytometry was used to quantify apoptosis (annexin V positive and PI negative) at early (20 hours) and late (48 hours) time points after hMPV infection. In cells exposed to FP, with or without hMPV infection, there were significantly fewer apoptotic cells 20 hours after infection, compared with cells not exposed to GCS (Figure 3A and Supplementary Figure 5). Next, caspase activities were examined, showing that caspase-3/7 activity was significantly decreased in PBECs exposed to GCS compared with both hMPV-infected and uninfected cells at 20 hours after infection. The addition of RU486 blocked this reduction (Figure 3A).

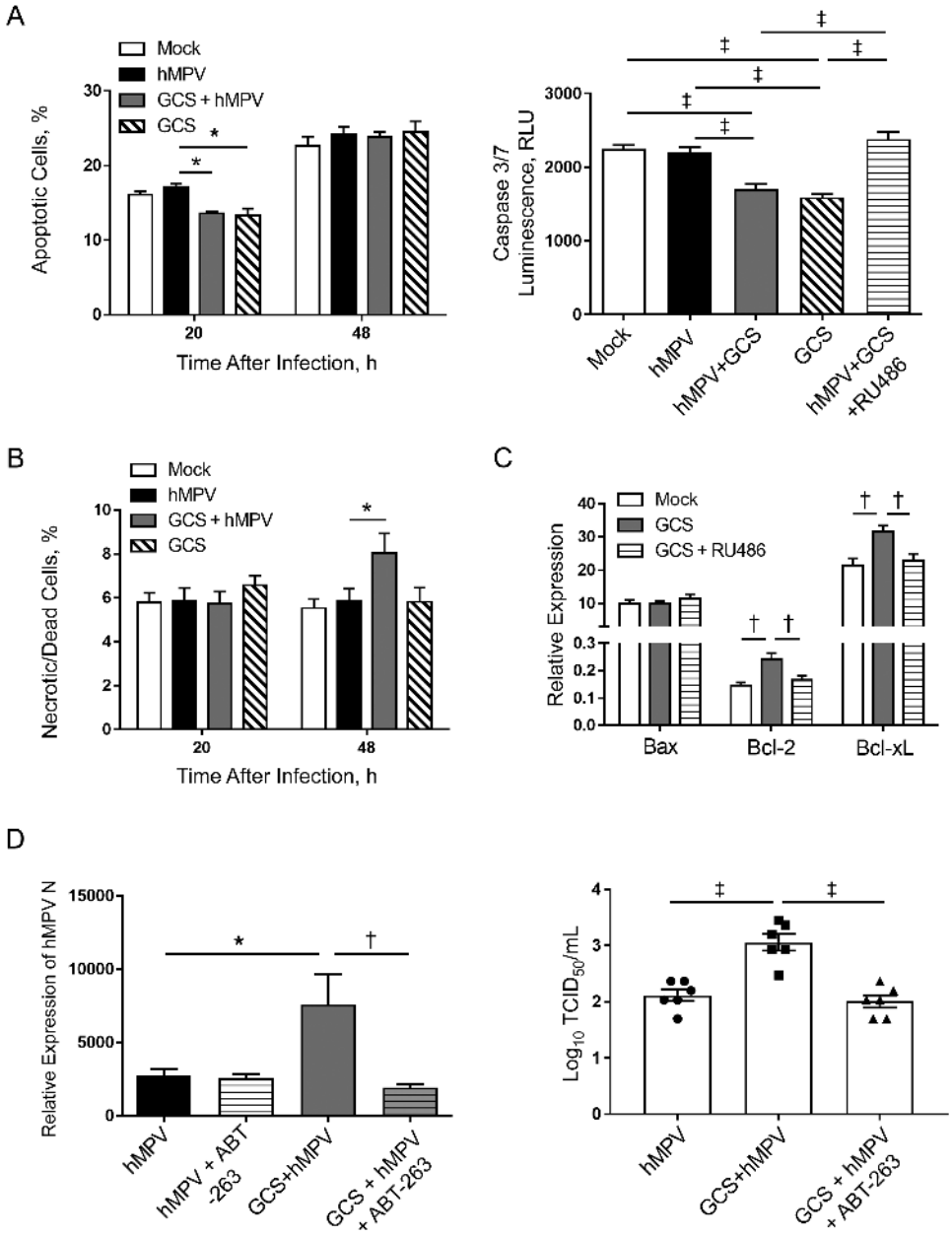

Figure 3. Glucocorticosteroid (GCS) treatment increases human metapneumovirus (hMPV) replication by inhibiting apoptosis. *A–C*, Human primary bronchial epithelial cells (PBECs) from healthy individuals pretreated with or without GCS were infected with hMPV (multiplicity of infection [MOI], 1). *A*, Apoptosis was analyzed by flow cytometry and caspase 3/7 activity. RLU, relative light units. *B*, Necrotic/dead cells were identified by means of flow cytometry. *C*, PBECs treated with or without RU486 were treated with GCS for 5 hours, RNA extracted from cells, and proapoptotic (Bcl-associated X protein [Bax]) and antiapoptotic (B-cell lymphoma [Bcl]-2 and Bcl-xL) gene expression was determined by reverse-transcription polymerase chain reaction (RT-PCR) and normalized to 18S. *D*, PBECs pretreated with or without GCS or ABT-263 were infected with hMPV (MOI, 1). RNA was extracted from cell monolayers at 48 hours after infection and hMPV nucleocapsid (hMPV N) gene expression was determined by RT-PCR and normalized to 18S and viral titers of culture supernatants were determined by plaque assay. Data represent means with standard errors of the mean for 3 replicate samples from 3 independent donors. $TCID_{50}$, median tissue culture infective dose. Comparisons were analyzed using 1- or 2-way analysis of variance, as appropriate. $^{*}P < .05$; $^{\dagger}P < .01$; $^{\ddagger}P < .001$

Finally, flow cytometry was used to identify necrotic/dead cells (annexin V positive and PI positive) at early (20 hours) and late (48 hours) time points. At 48 hours after hMPV infection and exposure to GCS, there was a significant increase in the number of cells manifesting markers of necrotic cell death (Figure 3B and Supplementary Figure 5). These findings indicate that GCS may suppress apoptosis early, thereby blunting this mechanism of virus elimination. The excess replication of hMPV is then reflected by increased cell death and necrosis at the later time point (48 hours).

This putative mechanism is feasible because GCS induce both propoptotic (Bcl-associated X protein [Bax]) and antiapoptotic genes (B-cell lymphoma [Bcl]-2 and Bcl-xL) [24]. We therefore examined the effect of GCS on gene expression of Bax,

Bcl-2, and Bcl-xL in PBECs. As a control, cells were treated with RU486, and gene expression was analyzed 5 hours after GCS exposure. GCS significantly enhanced expression of the antiapoptotic genes Bcl-2 and Bcl-xL, and the increase was absent if RU486 was used (Figure 3C). Finally, ABT-263, a specific inhibitor of the antiapoptotic proteins Bcl-2 and Bcl-xL [25], was added to PBECs with or without GCS treatment. ABT-263 significantly decreased hMPV N gene expression and viral titers in PBECs treated with GCS to levels equivalent to untreated PBECs (Figure 3D).

Recombinant IFN Treatment to Modulate hMPV Infection

Using both in vitro and murine models we have demonstrated that GCS significantly reduce expression of innate antiviral IFN, thereby increasing RV and influenza A virus replication. Adjuvant IFN blunted the detrimental effects of GCS [16]. Although endogenous IFN was not reduced by GCS in the hMPV infection model, it is possible that further enhancement of IFN responses by exogenous IFN will permit a reduction of virus replication and inflammatory consequences of infection. PBECs from healthy subjects, asthmatic patients, and patients with COPD were infected with hMPV and then exposed to recombinant IFNs. Addition of IFN-α_2, IFN-β, or IFN-λ_1 to cells significantly reduced hMPV replication (Figure 4A–4C). PBECs were also exposed to GCS and infected with hMPV, after which recombinant IFNs were added. Again, a reduction in virus replication was observed despite GCS treatment (Supplementary Figure 6). It is of note that, as expected, recombinant IFN-λ was effective, because the type III IFN receptor is selectively expressed only on epithelial cells [26].

The advantages of recombinant IFN are evident, but it is not known whether benefits include reductions in airway inflammation caused by hMPV infection. This aspect is difficult to gauge using in vitro models, and we therefore conducted studies in an established BALB/c mouse model of hMPV infection [17]. BALB/c mice were infected with hMPV and treated with recombinant IFN-β on days 1 and 3 after infection. The mice were culled on day 5 for measurements of hMPV replication and analyses of lung inflammation. Treatment with IFN-β significantly reduced hMPV replication (viral titers) in the lung (Figure 5A). In addition, parameters of airway inflammation were markedly reduced, including attenuated infiltration of leucocytes into perivascular tissue, reduced mucus-secreting cells, and reduced epithelial inflammatory changes (Figure 5B–5D).

DISCUSSION

Worldwide recognition of hMPV infection has been facilitated by new highly sensitive molecular diagnostic methods in clinical virology. Our clinical studies show that hMPV was the second most common virus associated with hospitalized COPD exacerbations and detectable in 5% of patients. Investigations in PBECs, obtained from patients with COPD, as well as healthy human volunteers and asthmatics, demonstrate that hMPV replicates robustly and induces appropriate innate IFN responses. Replication and innate and inflammatory responses were enhanced in cells obtained from patients with COPD. Exposure to clinically relevant concentrations of GCS also enhanced hMPV replication, a process mediated by reduced apoptosis followed by increased cell necrosis. Finally, proof-of-concept murine studies using recombinant IFN markedly reduced hMPV replication and attenuated airway inflammation.

Our study of hospitalized COPD exacerbations confirmed the role of hMPV as a cause of intercurrent worsening of COPD, albeit in a small number of patients. We detected hMPV in 5% of a large prospective cohort, second only to RV in detection rate. These results accord well with comparable studies that detected hMPV in 2.3%–12% of patients with COPD exacerbations [3–6, 27] and corroborate the ubiquitous role of hMPV in exacerbations of COPD.

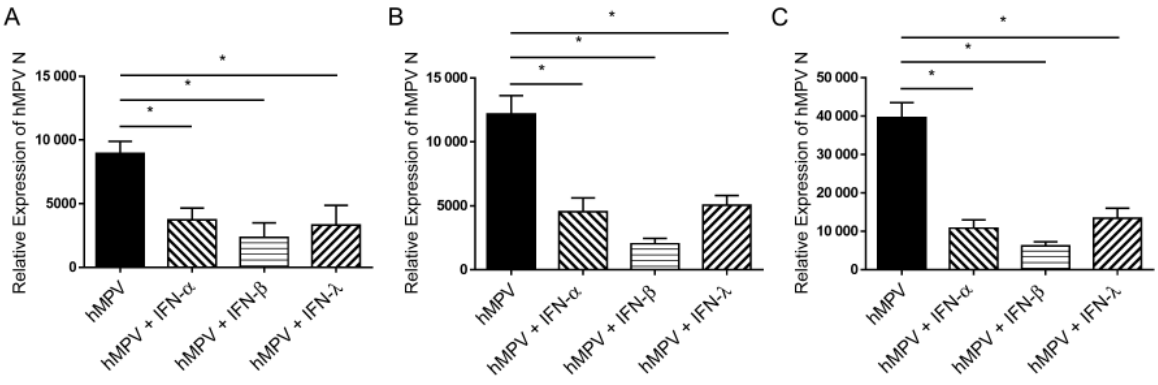

Figure 4. Interferon (IFN) treatment attenuates human metapneumovirus (hMPV) infection in vitro. Primary bronchial epithelial cells (PBECs) from healthy (*A*) and asthmatic (*B*) subjects and individuals with COPD (*C*) were infected with hMPV (multiplicity of infection [MOI], 1) and then treated with recombinant IFNs (α, β and λ) 1 hour after infection. RNA was extracted from cell monolayers at 72 hours after infection and reverse-transcription polymerase chain reaction (RT-PCR) was performed. hMPV nucleocapsid (hMPV N) gene expression was measured and normalized to 18S. Data are represented as means with standard errors of the mean for 3 replicate samples from ≥3 independent donors. Comparisons were analyzed using 1-way analysis of variance. *$P < .001$.

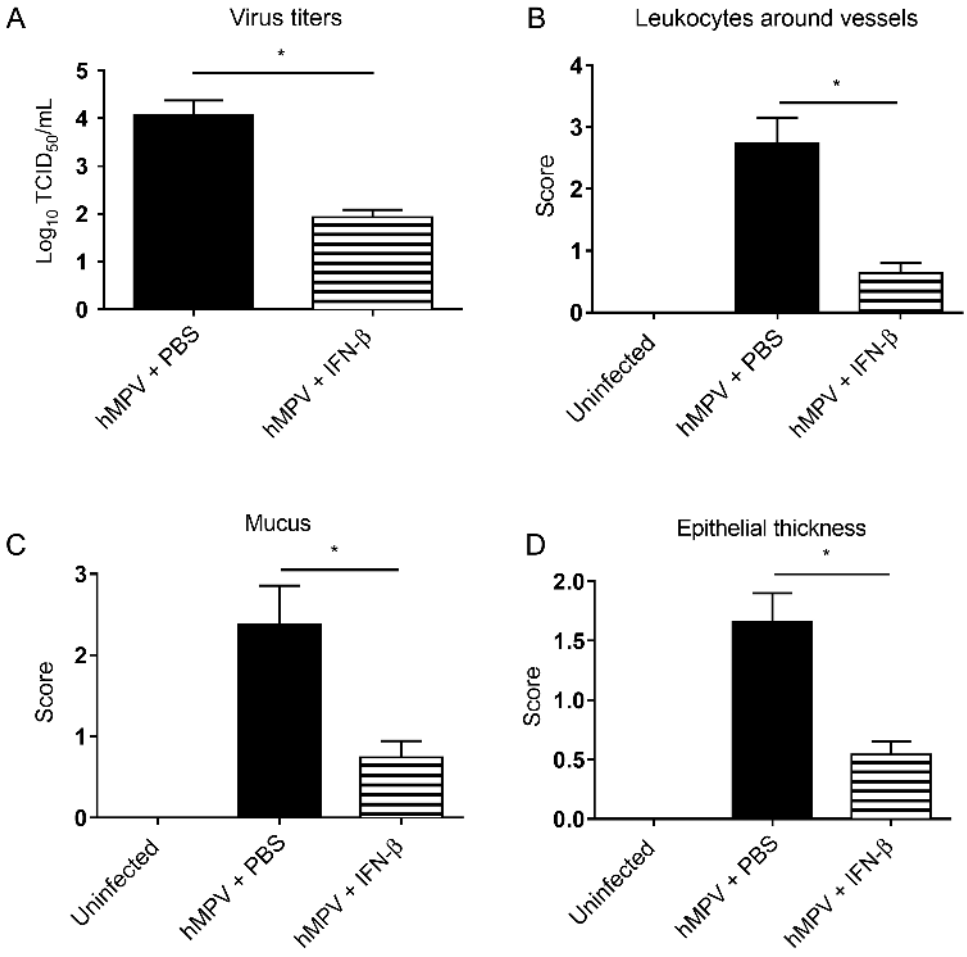

Figure 5. Recombinant interferon (IFN)-β reduces human metapneumovirus (hMPV) replication and airway inflammation in a mouse model of hMPV infection. Mice were infected intranasally with 10^6 plaque-forming units of hMPV and given intratracheal instillation of recombinant murine IFN-β or vehicle (phosphate-buffered saline [PBS]) at 1 and 3 days after infection. Mice were killed 5 days after infection and viral titers (*A*) and pulmonary inflammation (*B-D*) were evaluated. Data represent means with standard deviations for 2 experiments (3–5 mice per group). Analyses were conducted using an unpaired *t* test (for viral titers) or 1-way analysis of variance. *$P < .001$.

Human hMPV infection occurs after inhalation of the virus and adhesion to airway epithelial cells [28]. Intracellular replication is followed by release of virus after cell death, leading to infection of adjacent cells. The current understanding of this process is based mainly on animal models, and to date no studies have sourced human lower airway cells to investigate replication, key early immune responses and the consequences of commonly used inhaled GCS medications on hMPV infection. Our studies in epithelial cells obtained from healthy and asthmatic volunteers and those with COPD found that hMPV replicates robustly, eliciting innate IFN responses. Increased hMPV replication was observed in PBECs from patients with COPD, although there was no impairment of innate responses. The implications of this finding are unclear, and it is not known whether an increase in virus replication of this magnitude has clinical consequences.

In comparable studies, Spann et al reported enhanced virus replication in tracheal epithelial cells obtained from patients with atopy and wheezing, also with no impairment of IFN responses [18]. In this respect hMPV may differ from RV, the commonest cause of virus exacerbations. Current information suggests that RV may replicate more in the airways; chiefly as a result of impaired antiviral IFN responses [11, 20]. This appears to be different in hMPV and investigations are needed to understand the distinctive nuances of hMPV-associated virus-host interactions as well as to confirm and extend our findings in COPD.

We next evaluated key cytokine responses to hMPV infection mediated via the IFN and NF-κB pathways. After hMPV infection of PBECs, there was induction of IP-10 and RANTES, but IL-6 and IL-8 were not increased. This finding is similar to that of a previous study by Bao et al [29] demonstrating that a lack of vital NF-κB family cytokine responses after hMPV infection was due to the hMPV small hydrophobic protein inhibiting NF-κB transcriptional activity in airway epithelial cells and blocking cytokine production. Significant induction of IP-10 and RANTES were observed in COPD. Both cytokines are IRGs and are likely to reflect host IFN responses to hMPV. However,

IP-10 and RANTES also have strong proinflammatory activities and may contribute to inflammation after infection.

Inhaled GCSs are in widespread use in obstructive lung diseases, delivering effective anti-inflammatory treatment. This in turn translates into clinical benefits with significant effects on symptoms, exacerbation rates, and overall quality of life [30]. However, GCSs have a 'darker' side, and earlier studies have shown associations with pneumonia in patients with COPD or asthma [12, 13, 15]. This has been postulated to reflect immunosuppressive actions of GCS, which in turn lead to intensified virus and possibly bacterial infections.

The role of inhaled GCSs has become a key, unresolved question in the pharmacological management of COPD in particular. The clinical use of GCS was modeled in our studies, and we show that infection with hMPV in the presence of "therapeutic" levels of GCS increased hMPV replication. The effect of GCS was blocked by RU486, a molecule that antagonizes the GCS receptor, demonstrating specificity of the GCS effect. Surprisingly, GCS did not mediate enhanced virus replication by blunting IFN responses, as noted for other viruses [16], but rather by subverting apoptosis. A recent report also showed that hMPV replication was increased in nasal epithelial cells taken from asthmatic subjects and that this was mediated by reduced apoptosis via heat shock protein 70 [31].

Apoptosis is a cardinal host defense mechanism that eliminates virus, because programmed cell death leads to efficient virus death without excessive inflammatory consequences [32]. GCS treatment attenuated apoptosis, allowing cells to survive longer and permitting enhanced viral replication, with subsequent increased viral shedding from necrotic cells. We also show that GCS induced antiapoptotic genes Bcl-2 and Bcl-xL and that a GCS receptor antagonist and specific blocker prevented this activity. Some viruses cause necroptosis, programmed necrosis mediated through the receptor-interacting protein kinase 1–3 complex [33]. It is unclear whether cell death after hMPV infection was a result of necrosis or necroptosis, because PI staining does not differentiate between the 2 processes. The detailed mechanisms that underpin this finding merit further study.

Recombinant IFN-β in aerosolized format has recently been shown to reduce the severity of RV-induced exacerbations in a severe asthma subgroup [34]. In further proof-of-concept studies pertaining to hMPV infection, we show both in vitro and in a murine model that this approach may be equally applicable to hMPV. Exposure to IFN reduced hMPV replication significantly and also blunted the effects of GCS on virus replication in PBECs. In mice, IFN-β delivered via the trachea yielded a marked reduction in viral load but also significantly reduced airway inflammation resulting from infection. IFN-β treatment was therefore highly protective, which indicates that recombinant aerosolized IFNs are likely to have broad utility to protect patients with COPD from detrimental consequences of virus infections, probably irrespective of the virus species.

In summary, robust hMPV infection occurs in human bronchial epithelial cells, and this may be more severe in COPD. Increased replication of hMPV associated with GCS treatment can be countered by use of recombinant IFN. Further studies are needed to clarify the pathogenesis of hMPV; this will aid development of antiviral therapies and inform vaccination strategies. Use of aerosolized IFN to counter intercurrent virus infections can potentially have significant benefits in respiratory diseases.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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